Distribution and Survival of *Escherichia coli* Translocating from the Intestine After Thermal Injury

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The present investigation was performed to study the kinetics of tissue distribution and deposition of *Escherichia coli* and endotoxin translocating from the intestine after thermal injury. *Escherichia coli* was grown in the presence of ^{14}C glucose and both labeled bacteria and endotoxin prepared from the labeled bacteria were used as translocation probes. *Escherichia coli* (10^8 to 10^{10} bacteria) and *E. coli* endotoxin (100 μg per animal) were gavaged into the stomach immediately before a 30% burn injury was inflicted in mice. Animals were killed 1, 4, and 24 hours after burn injury. Translocation occurred extensively within 1 hour after burn injury. Expressed as amount of radioactivity per gram of tissue, translocation was greatest in the mesenteric lymph node (MLN) followed by spleen, lung, and liver. Translocation of endotoxin was similar to translocation of intact bacteria, with the exception that less radioactivity could be found in the peritoneal cavity and more in the liver. Both intact *E. coli* and endotoxin translocated directly through the intact bowel wall. Killing of bacteria was greatest in the MLN and spleen, approximating 95% to more than 99% of translocating bacteria. Killing efficiency was lowest in the lungs. It is concluded that estimation of translocation by viable bacterial counts in tissues grossly underestimates the extent of translocation of bacteria and ignores the extent of translocation of endotoxin. Translocation of endotoxin may have biologic significance that is independent of and in addition to translocation of intact bacteria.

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MICROBIAL TRANSLOCATION, DEFINED as the passage of both viable and nonviable microbes and microbial products, including endotoxin, across an anatomically intact intestinal barrier, has become a fashionable topic of surgical research. This process could be, at least in part, a predisposing factor for systemic infections,[1-4] postinjury hypermetabolism,[5,6] multiple-system organ failure in seriously ill patients,[7] and clinical sepsis in the absence of a defined focus of infection.[8,9] Studies in experimental animals revealed that translocation as measured by recovery of viable organisms from regional lymph nodes is increased by agents causing direct injury of mucosal cells and by indirect injury, which is almost always associated with reduced blood flow.[10-12] In addition diseases associated with ulceration of the gastrointestinal tract are associated with recovery of viable organisms from the mesenteric lymph nodes.[10,11] Several predisposing factors for translocation have been identified, including alteration of the intestinal flora, malnutrition, immune compromise, and very young age. Hemorrhagic shock and thermal injury with malnutrition served as useful models for studying translocation. However the 'translocation' reported to occur in models of hemorrhagic shock appears to be primarily through mucosal ulcerations, whereas in models of burn injury and malnutrition, there is clear evidence for direct passage of both organisms and endotoxin through morphologically intact enterocytes.[13,14]

Because most studies of microbial translocation have been quantitated by the recovery of viable organisms from the mesenteric lymph nodes and other tissues by culture techniques, the relative rates of translocation and distribution of organisms that are killed by host defense mechanisms and the rate and distribution of translocation of endotoxin are largely unknown. The present investigation was performed to study the kinetics of tissue distribution and deposition of *Escherichia coli* and *E. coli* endotoxin translocating from the intestine after thermal injury.

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Materials and Methods

Animals and Animal Care

Adult female BALB/c mice weighing 20 to 22 g (Charles River, Wilmington, MA) were used for all experiments. They were caged in groups of five and provided food (Rodent Laboratory Chow 5001, Purina Mills, Inc., St. Louis, MO) and water *ad libidum* during a quarantine period of 1 week so they could adapt to the standard laboratory environment and to detect any pre-existing diseases. The protocols were approved by the University of Cincinnati Medical Center's Institutional Animal Care Use Committee and the animals were housed in an AAALAC-approved facility. All investigations adhered to the Guide for the Care and Use of Laboratory Animals as set forth by the Committee on the Care and Use of Laboratory Animals, National Research Council, the United States Department of Health and Human Services and the National Institutes of Health.

Preparation of Bacteria and Endotoxin

Escherichia coli (53104) was inoculated into glucose-free minimal nutrient media and grown for 19 hours at 37°C. ^{14}C glucose, 500 μCi (New England Nuclear, Boston, MA) was added to the suspension and incubated overnight. The culture was centrifuged to pellet the organisms, washed, quantitated with a Klett densitometer (Klett Mfg. Co., New York, NY), and adjusted to the desired concentration. Endotoxin was prepared and extracted from similarly labeled organisms by a previously described technique.[14]

Surgical Procedures and Burn Injury

One day before burn injury, the hair of the animal torsos was removed by clipping. Food was withheld for 18 hours but water was provided ad libidum before gavage with 0.1 mL of the translocation probe (^{14}C *E. coli* or ^{14}C endotoxin) or saline solution while the animals were awake. After gavage they were anesthetized with methoxyflurane inhalation anesthetic and a 30% full-thickness burn was inflicted using the technique of Holder et al.[15] Saline (0.1 mL) was administered immediately after burn injury for fluid resuscitation and the animals were allowed to recover from anesthesia. Groups of animals were reanesthetized at 1, 4, and 24 hours after burn, bled by cardiac puncture, and killed. Animals to be killed at 24 hours were allowed free access to food and water 4 hours after burn. Saline (1.0 mL) was injected into the peritoneal cavity and the abdomen was gently massaged to create even distribution of the fluid. The animals were prepped with 70% alcohol and aseptic techniques were used to remove the peritoneal fluid, mesenteric lymph nodes (MLN), spleen, liver, and lungs. The solid tissues (MLNs, spleen, liver, lungs) were weighed individually and homogenized after the addition of 1.0 mL of sterile normal saline. One hundred microliters of the homogenate was removed and plated on brain heart infusion (BHI) agar plates (Baltimore Biological Laboratories, Baltimore, MD) for quantatative colony counts after 18 hours of incubation. The remaining portion of the homogenate was lyophilized and clarified using a nitric acid procedure.[16] This solution was diluted 1:11 in 0.75 mol/L (molar) TRIS buffer and the disintegrations per minute (dpm) of ^{14}C in 0.5 mL was determined by liquid scintillation counting (Beckman Model LS 3133 liquid scintillation counter, Beckman Instruments Inc., Fullerton, CA). Blood was processed similarly through the decolorization procedure.

The viable colony counts were enumerated by counting and expressed as bacteria per gram of tissue or milliliter of fluid by multiplying the actual counts by $10 \times \frac{1 + \text{weight}}{\text{weight}}$. The radioactivity in tissues and fluids was expressed as dpm per gram of tissue after correction for dilution. Repeated experiments determined that approximately 1 dpm represented 121 organisms as measured by a Klett densitometer. Sample checks determined that counts by Klett densitometer approximated viable counts. Where applicable the percentage of viable bacteria in tissues and fluids was derived by dividing the observed viable bacteria by the number of estimated bacteria as determined by radioactivity in the tissues.

To determine the number of organisms that remained viable during intestinal transit, the stomach, small intestine, and colon were excised after aseptic removal of the MLNs, liver, spleen, and lungs. Each segment was washed with 1.0 mL sterile saline and the fluid was subjected to liquid scintillation counting and plate quantitation of colony counts on BHI plates.

Experimental Groups

Five experiments using 15 mice each were performed. Mice in experiment 1 were gavaged with 10^8 *E. coli* organisms per mouse, mice in experiment 2 with 10^9 organisms per mouse, and mice in experiment 3 with 10^{10} organisms per mouse. In experiment 4, mice were gavaged with 0.1 mL saline containing 100 μg of ^{14}C endotoxin. Experiment 5 was a duplicate of experiment 3. Mice were killed in groups of five 1, 4, and 24 hours after burn for determination of viable colony counts and nuclide counts in MLNs, spleen, liver, lungs, peritoneal fluid, blood, and washes from intestinal segments as described above.

Preliminary Experiments

Initial groups of animals had food withheld for 0, 6, 12, 18, and 24 hours before burn injury. The translocation rate of 10^{10} gavaged ^{14}C *E. coli* was highest in animals in which food was withheld for 18 or 24 hours, but animals

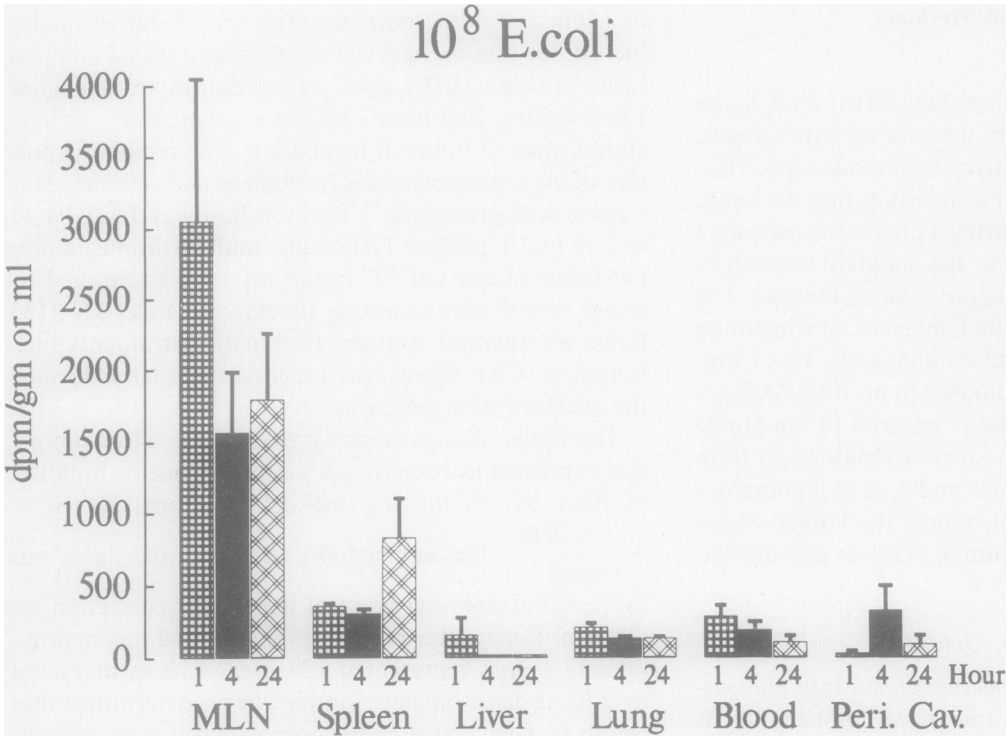

FIG. 1. Distribution of *E. coli* in tissues 1, 4, and 24 hours after gavage with 10^8 *E. coli* and burn injury as measured by dpm/g tissue or milliliters of fluid. All data in this and subsequent experiments are expressed as mean ± SEM.

deprived of food for 24 hours had a higher mortality rate. Therefore 18 hours of food deprivation was used for subsequent experiments.

Killing of animals at 1, 2, 4, and 6 hours after burn injury determined that translocation to tissues was well established by 1 hour and reached a peak no later than 4 hours. Translocation did not occur in animals gavaged with 10^{10} ^{14}C *E. coli* that were not burned.

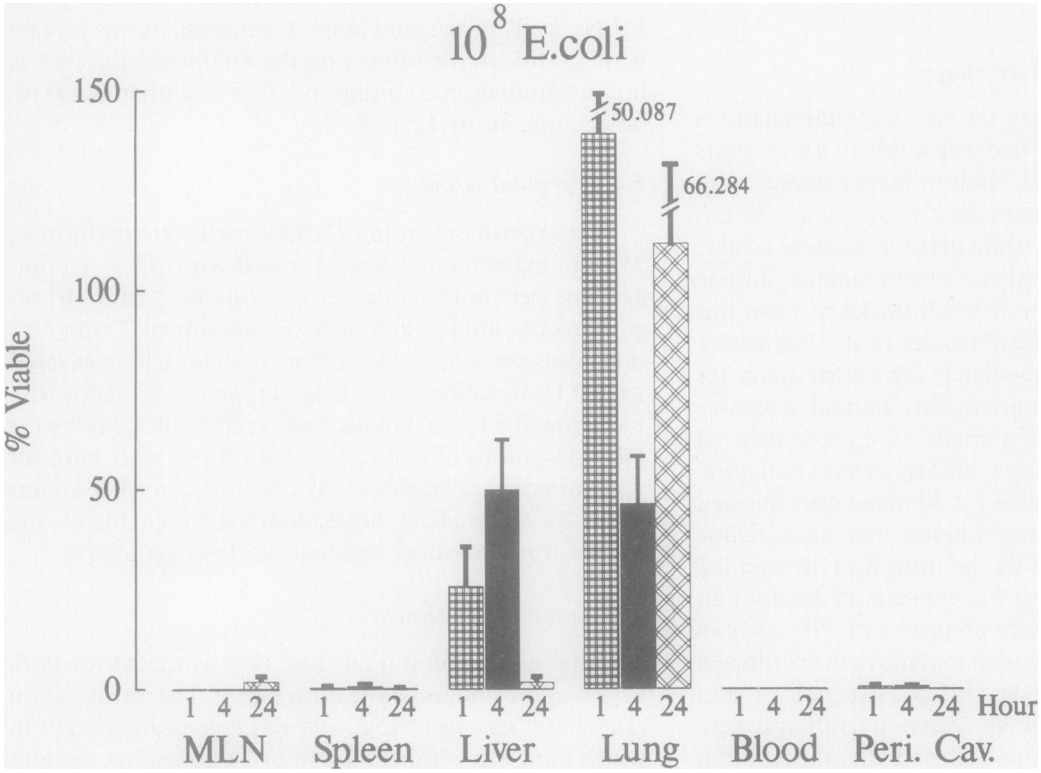

FIG. 2. The percentage of viable organisms that translocated to tissues in mice gavaged with 10^8 *E. coli* before burn.

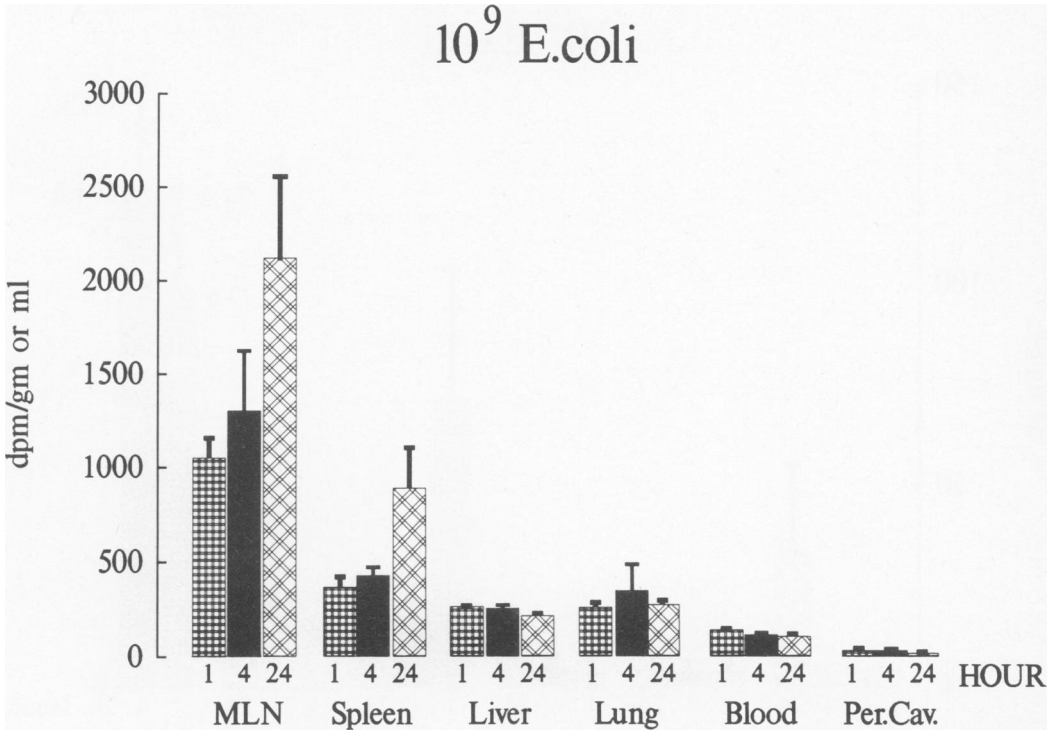

FIG. 3. Distribution of *E. coli* in tissues 1, 4, and 24 hours after gavage with 10^9 *E. coli* and burn injury as measured by dpm/g tissue or milliliters of fluid.

To determine the transit time through the intestine, animals were deprived of food for 18 hours, gavaged while awake with 0.1 mL India ink, anesthetized, burned, and killed at 1 and 4 hours after burn. By 1 hour the small intestine and cecum were filled with the India ink but none appeared in the feces. By 4 hours both feces and urine were darkly stained, as was the ileum, cecum, and colon. There was residual staining in the stomach. None of the animals had India ink grossly apparent in their lungs.

Experiment 1

The distribution of translocation as measured by radioactivity expressed as dpm per gram of tissue or milliliter of fluid is shown in Figure 1. The most concentrated activity was found in the MLNs, followed by the spleen and blood. When the percentage of organisms that remained viable was calculated for each tissue, more than 95% of those in the MLNs, spleen, blood, and peritoneal cavity were killed at each time interval. In contrast a greater percentage of organisms remained viable in the liver and, especially, the lungs (Fig. 2).

Experiment 2

Gavage with 10^9 *E. coli* resulted in a modest increase in the organisms recovered from all tissues, as shown by dpm per gram of tissue (Fig. 3). The same pattern of distribution was seen with the greatest numbers of counts found in the MLNs and spleen. When the percentage of viable organisms was calculated, similar trends were seen, generally with less than 5% of organisms surviving in the MLNs, spleen, and blood compared to higher viability in the lungs and peritoneal cavity (Fig. 4).

Experiment 3

When 10^{10} *E. coli* were gavaged, increased radioactivity was found in all of the tissues, but this was not proportionate to the increase in dose as compared to experiments 1 and 2. The pattern of distribution was similar to the previous experiments, with greater amounts translocating to the MLNs and spleen than to the liver or lung, which, in turn, were greater than the blood and peritoneal cavity (Fig. 5). Similar to experiment 2, the viability of organisms was greatest in the lungs and peritoneal cavity, with a greater percentage being killed in liver, spleen, MLNs, and blood, respectively (Fig. 6).

Experiment 4

In this experiment, animals were gavaged with 100 μg of ^{14}C endotoxin with a specific activity of 25,426 dpm per microgram. Judging from the tissue counts, shown in Figure 7, less than 0.1% of the administered radioactivity was found in the tissues at any given time, with the highest concentrations found in the MLNs, liver and lung, a somewhat different pattern from that seen for intact *E. coli*. The highest tissue endotoxin concentration, *i.e.*, in the MLNs represents approximately 0.04 μg/g tissue.

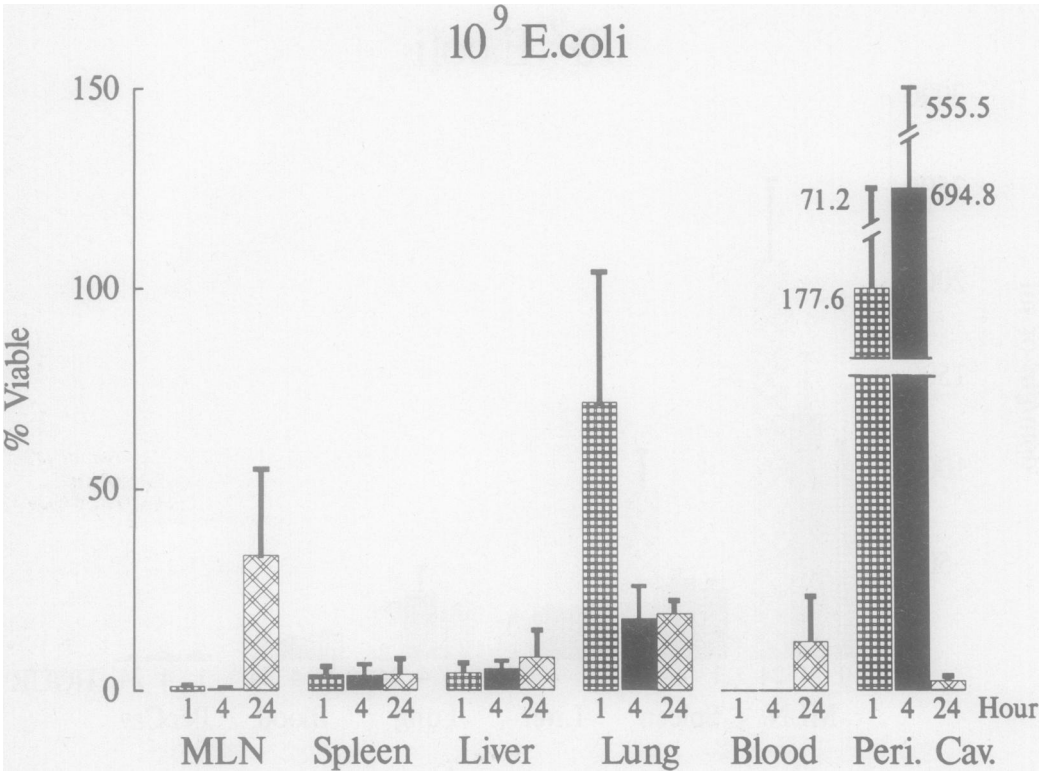

FIG. 4. The percentage of viable organisms that translocated to tissues in mice gavaged with 10^9 *E. coli* before burn.

Experiment 5

This experiment was a duplicate of experiment 3 and the data, presented in Table 1 in tabular rather than graphic form, generally confirm the findings of experiment 3, although the radionuclide counts were somewhat higher in MLNs and spleen, with a greater percentage of bacteria killed in these tissues.

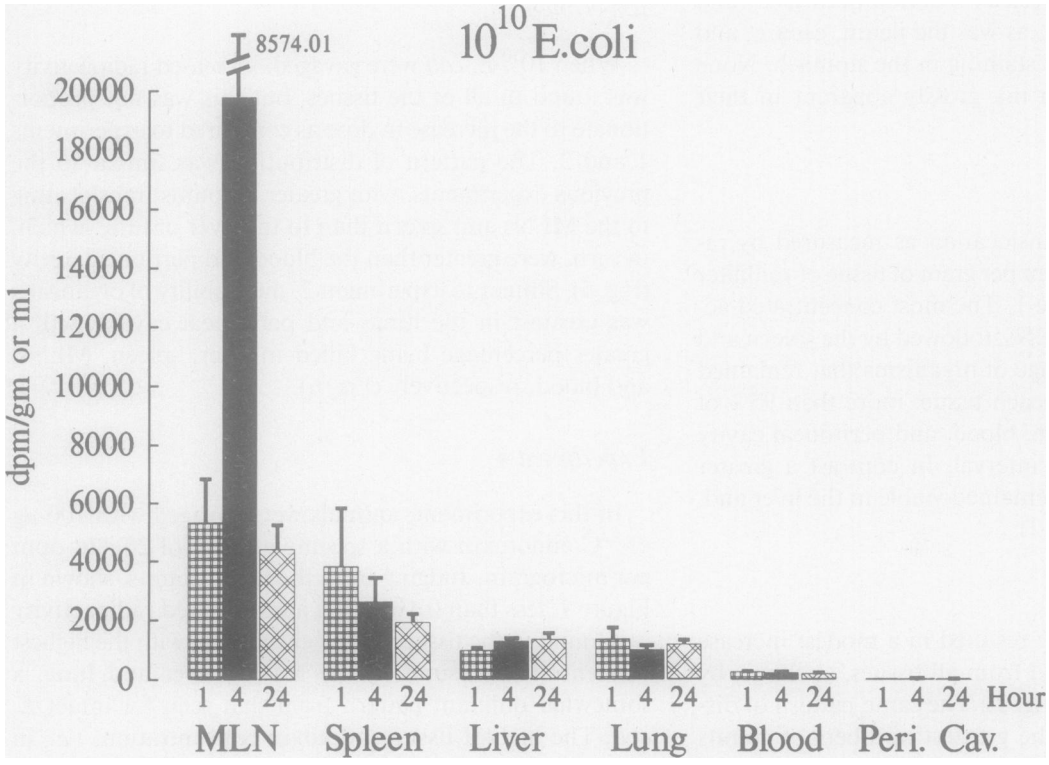

FIG. 5. Distribution of *E. coli* in tissues 1, 4, and 24 hours after gavage with 10^{10} *E. coli* and burn injury as measured by dpm/g tissue or milliliters of fluid.

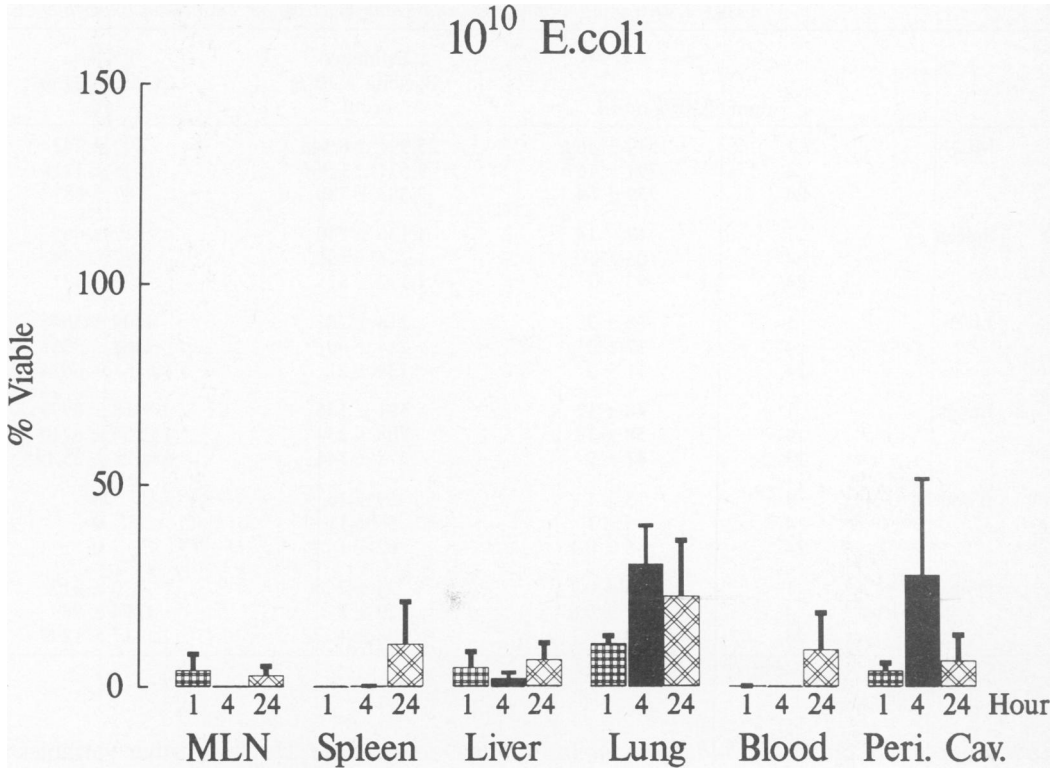

FIG. 6. The percentage of viable organisms that translocated to tissues in mice gavaged with 10^{10} *E. coli* before burn.

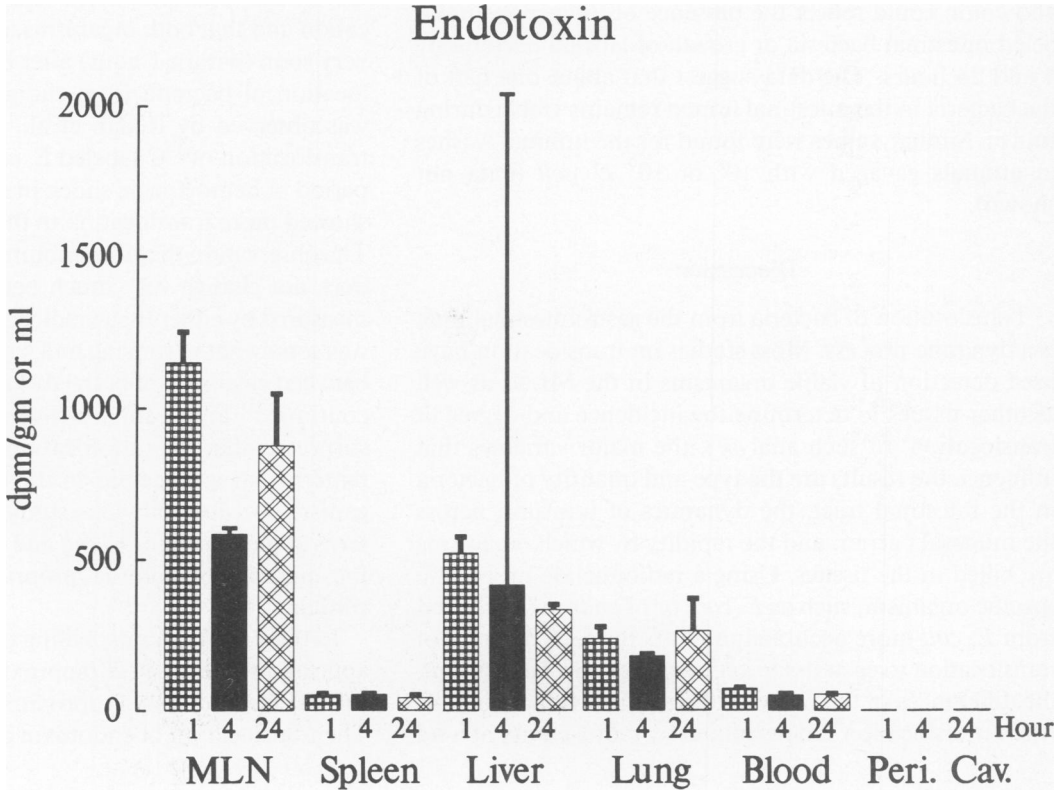

FIG. 7. Distribution of endotoxin in tissues 1, 4, and 24 hours after gavage with 100 μg ^{14}C endotoxin and burn injury as measured by radionuclide activity.

TABLE 1. *Distribution of Nuclide and Viable Bacteria for Animals in Experiment 5*

		dpm × 10^2/g or mL	Estimated Bacteria × 10^3/g or mL	Bacteria Cultured/g or mL	Percentage of Viable Bacteria
MLN	1	1519 ± 469	23,997 ± 6,145	400 ± 242	0.007 ± 0.001
	4	791 ± 157	9,571 ± 1,906	1548 ± 1116	0.01 ± 0.007
	24	279 ± 64	3,383 ± 780	787 ± 681	0.29 ± 0.26
Spleen	1	117 ± 14	1,136 ± 260	817 ± 665	0.19 ± 0.21
	4	105 ± 10	1,275 ± 106	827 ± 579	0.07 ± 0.04
	24	93 ± 7	1,132 ± 87	498 ± 431	0.04 ± 0.04
Liver	1	18 ± 3	218 ± 28	4404 ± 3645	2.08 ± 1.67
	4	17 ± 3	214 ± 40	7468 ± 3353	3.63 ± 1.46
	24	11 ± 2	134 ± 21	10,167 ± 6674	3.80 ± 2.18
Lungs	1	48 ± 12	591 ± 146	37,918 ± 8998	5.56 ± 1.26
	4	58 ± 19	708 ± 234	15,254 ± 6710	11.63 ± 3.74
	24	41 ± 9	378 ± 144	68,628 ± 35,145	20.08 ± 12.14
Blood	1	5 ± 1	59 ± 16	0	0
	4	5 ± 1	56 ± 11	0	0
	24	5 ± 0.1	62 ± 1	0	0
P wash	1	3 ± 0.7	34 ± 7	870 ± 517	4.96 ± 3.61
	4	2 ± 0.6	29 ± 8	1294 ± 989	4.73 ± 2.21
	24	3 ± 0.1	38 ± 1	1317 ± 1297	3.20 ± 3.15

Values are given as mean ± SEM.

Determination of Bacterial Viability in the Intestinal Tract

The washes of the contents of the stomach, small intestine, and colon are shown in Figure 8 as a percentage of viable bacteria related to radionuclide counts. Percentages greater than 100% found in the small intestine and colon could reflect the presence of indigenous unlabeled intestinal bacteria or growth of labeled bacteria by 4 and 24 hours. The data suggest that about one half of the bacteria in the intestinal lumen remains viable during transit. Similar values were found for the luminal washes in animals gavaged with 10^8 or 10^9 *E. coli* (data not shown).

Discussion

Translocation of bacteria from the gastrointestinal tract is a dynamic process. Most studies on translocation have used detection of viable organisms in the MLNs as well as other tissues to determine the incidence and degree of translocation. In such analyses, the major variables that influence the results are the type and quantity of bacteria in the intestinal tract, the dynamics of transport across the mucosal barrier, and the rapidity by which organisms are killed in the tissues. Using a radionuclide probe of a specific organism, such as *E. coli*, or of endotoxin derived from *E. coli* more accurately reflects the total amount of translocation to each tissue and removes, to a large extent, the differences in the rate of killing by individual animals when translocation is determined by radioactivity at a set time. However other variables are introduced, including multiplication of organisms both in the intestinal tract and tissues and redistribution of the isotope by metabolic processes of the organism and of the phagocytic cells after ingestion. The present studies suggest that perhaps one half of the organisms were viable at the time of translocation and that both organisms and endotoxin translocate very soon (within 1 hour) after burn injury. Rapid translocation of bacteria from the gastrointestinal tract also was observed by Redan et al.,[17] who showed extensive translocation of ^{14}C-labeled *E. coli* by the end of a 5-hour period of hemorrhagic shock in rats. However their study showed more translocation to the lung than to the spleen. The observation that the amount of translocated organism does not change very much between 1 and 24 hours as measured by either tissue radioactivity or viable organisms was somewhat surprising and suggests a dynamic balance between clearance of both label and viable organisms and continued translocation through the enterocytes. It is not surprising that the translocation of endotoxin followed a pattern that was similar to the distribution of intact organisms because previous studies suggested that the patterns of movement of *E. coli* and *E. coli* endotoxin through the enterocytes, lamina propria, and muscularis were similar.[14]

In these experiments, killing of translocated organisms appeared to be greatest (approximately 99% or more) in the MLNs and least (approximately 50%) in the lungs. The translocation of endotoxin appeared to be somewhat

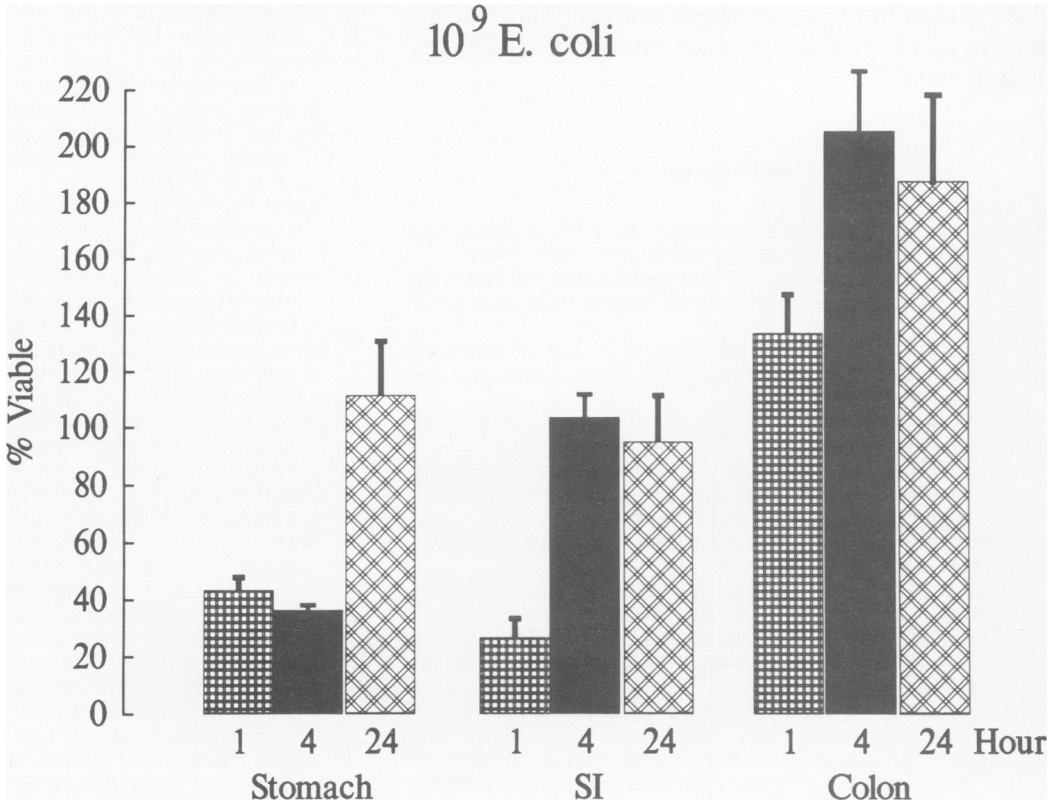

FIG. 8. The percentage of viable organisms in the stomach, small intestine, and colon as determined by actual colony counts divided by bacteria estimated from radionuclide activity is for animals gavaged with 10^9 *E. coli* before burn injury. The increase in percentage of viable bacteria after 1 hour in the stomach and small intestine could represent growth of labeled organisms, whereas the presence of indigenous organisms could account for high counts in the colon.

different from intact *E. coli* because there was more translocation to the liver and less to the peritoneal cavity. Because it is water soluble and often bound to proteins, it may be that the endotoxin that translocated to the peritoneal cavity was absorbed systemically more easily than the labeled *E. coli* trapped by peritoneal macrophages or neutrophils. Both the experiment with the endotoxin and the viable *E. coli* indicate that the biologic effects of endotoxin translocation may be much greater than could be appreciated by measurement of colony counts in the MLNs or other tissues. The concentration of endotoxin detected in tissues (up to 0.04 μg/g) is more than that necessary to stimulate macrophages and other cells *in vitro*. Because only 100 μg of endotoxin was administered and endotoxin concentrations may considerably exceed this in stool, it is very likely that endotoxin translocation is of great biologic importance independent of translocation of intact organisms. Endotoxin translocation has been shown previously by Rush et al.[18] using a hemorrhagic shock model in germ-free rats as well as others using conventional animals.[19-21] *In vitro* infection studies by Nolan et al.[22] indicated that endotoxin movement across the enterocyte was an active process involving a transport system.

These studies suggest that the use of a translocation probe, *i.e.*, the administration of a known quantity of a radionuclide-labeled bacteria or bacterial product, may be a superior way to assess differences in translocation rates across the mucosal barrier when comparing different experimental groups. An intragastric inoculum of approximately 10^{10} *E. coli* appears to be a suitable dose in this animal model. The technique has other advantages in that the relative contributions of the rate of translocation across the mucosal barrier and the rate of microbial killing by host defense mechanisms can be assessed simultaneously.

Translocation of intact organisms into the peritoneal cavity through the intact intestinal wall was shown by previous investigators, including Wells et al.[23] and Mora et al.,[24] who demonstrated that enteric organisms could be found in monocontaminated experimental intra-abdominal abscesses and intra-abdominally implanted foreign materials, respectively. Earlier studies by Papa et al.,[25] and by Booth et al.[26] suggested migration of intact bacteria through an anatomically intact intestinal wall in the presence of ischemia or obstruction. Maejima and Deitch[27] and Deitch et al.[28] also showed translocation of bacteria to the peritoneal cavity after burn injury in mice. The precise process by which organisms pass directly through the muscularis remains to be determined, although pre-

vious studies from our laboratory showed that both endotoxin and *E. coli* pass between mycocytes rather than through them.[14]

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Discussions

DR. BEN RUSH (Newark, New Jersey): Part of my enjoyment of this paper is the chance to see some of our previous work confirmed and extended in a different model.

While there are some differences in detail between our study and Dr. Alexander's, the thrust of the two studies is much the same. Dr. Alexander has added exciting new data in quantitating the percentage of viable bacteria and the translocation of endotoxin.

As little as 3 or 4 years ago, bacterial translocation was not an accepted concept. Now it is widely accepted as a common phenomena in various types of trauma. What we now must do is quantitate its role.

My question to Dr. Alexander is, does he think this is an epiphenomena that is observed in burns but has no special effect, or is this a central part of the septic response?

DR. EDWIN DEITCH (Shreveport, Louisiana): The concept, that bacterial translocation occurs has become more accepted. Now we must focus on two questions. One is its relevance to clinical practice and the other is the mechanisms that promote and limit translocation.

As you know, we have been interested in this field for quite a while and have noticed some very fascinating observations, one of which, I think, focuses on the study that Dr. Alexander showed us today. And that is the concept that bacterial translocation is not an all or none phenomenon. We see conditions in which translocation occurs, yet the animal appears perfectly well, and in which translocation appears to be a microbiologic phenomenon. On the other hand, we see conditions in which bacteria or endotoxin ultimately can lead to gut origin sepsis, death, and a septic status.

Thus we need to understand what factors limit the systemic spread of bacteria once barrier function is lost. We believe that it is the immune system that is important. One loses barrier function, the bacteria cross; if the immune system is normal, bacterial spread is limited, but if the immune system is impaired, the bacteria may spread systematically.

Because of that I think the work that was presented today is exceptionally important in putting the ability of the host to control bacteria in perspective.

Have you done any immunologic studies to determine what it is that allows these bacteria to survive and, conversely, what it is that allows these bacteria to be killed?

The concept of the multi-insult model, I think, applies here because